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## Pharmacognostical and phytochemical study of apamarga tandulasa (seeds of *Achyranthes aspera* L.)

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### Abstract

Apamarga tandula described in ayurvedic samhitas like Charak samhita, Susruta samhita, Astang hridaye etc. It is very important ayurvedic medicinal plant. Which is used in different diseases like obesity, jaundice etc. It is also used in making of ksharsutra in ayurveda. In the present study different parameters used for the analysis of seeds of *Achyranthes aspera* L. like Determination of foreign matter, Loss on drying, Total ash value, Acid insoluble ash hydro-alcoholic extractive value, pH, Qualitative Phytochemical Screening, UV-Vis spectroscopy.

**Keywords:** *Achyranthes aspera* L., Loss on drying, total ash value, UV-Vis spectroscopy

### Introduction

*Achyranthes aspera* L. Plant is well known dravya in Ayurveda. It has mentioned in vedic compendia as well as in Brihatrayai and later on, it has been described in nighantus. In present work, preliminary phytochemical and pharmacognostical study on seeds of *Achyranthes aspera* L. has been done.

- 1. Collection-** Seeds of *Achyranthes aspera* L. was collected from local market of Varanasi.
- 2. Identification-** Seeds of *Achyranthes aspera* L. was identified by Prof. Anil kumar singh, Department of Dravyaguna in BHU.



**Fig 1:** Plant of *Achyranthes aspera* L.



**Fig 2:** Seeds of *Achyranthes aspera* L.

### 3. Pharmacogonosomal Description

#### Macroscopically Characters

Macroscopic observation of seeds of *Achyranthes aspera* L. was done. It comprised of shape, size, surface characteristics, texture, color, consistency, taste, etc.

#### 4. Physico-Chemical Analysis <sup>[1]</sup>

##### a. Determination of foreign matter

100 g sample was accurately weighed and spreader in a thin layer and sorted the foreign matter into groups either by visual inspection or by using a magnifying lens (6X or 10X). The remainder of the sample was shifted through a no. 250 sieve; dust was regarded as mineral admixture.

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**b. Determination of loss on drying**

10 g of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish. It was dried at 105°C for 5 hours and at 1 hour interval until difference two successive weightings.

**c. Determination of Total ash value**

2 gram of sample was accurately weighed in a tarred silica crucible at temperature 450 °C until it was free from carbon. Then it was cooled and weighed. The percentage of total ash was calculated with reference to the air- dried drug.

**d. Determination of Acid insoluble ash**

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter obtained was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried drug.

**e. Water-soluble Ash**

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at temperature 450°C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water- soluble ash was calculated with reference to the air dried drug.

**g. Determination of hydro-alcoholic extractive value**

Hydro-alcoholic extract of air dried 100 gm coarse powder of the sample was extracted with Ethanol: Distilled water (50:50), 450 ml each, with continuous heat extraction with Soxhlet apparatus and filtered. The extract was concentrated to get dry residue and stored in the desiccators and after that the percentage of hydro-alcoholic extract was calculated with reference to the air dried drug.



**Fig 3:** Soxhlet apparatus during extraction process



**Fig 4:** Hydro-alcoholic Extract.

**h. Determination of pH**

The powder of sample was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours at room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated pH meter.

**5. Qualitative Phytochemical Screening <sup>[2]</sup>**

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.

**a. Alkaloids**

- **Dragendorff's test:** Dissolve a few mg of hydro-alcoholic extract until an acid reaction occurs, and then add 1 ml of Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.
- **Hager's test:** 1 ml of Hydroalcoholic extract of the drug was taken in a test tube, adding a few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.
- **Wagner's test:** Acidifying 1 ml of hydro-alcoholic extract of the drug with 1.5% w/v of hydrochloric acid and adding a few drops of Wagner's reagent. A yellow or brown precipitate is formed.
- **Mayer's test:** Adding a few drops of Mayer's reagent to 1 ml of hydro-alcoholic extract of the drug. White or pale yellow precipitate is formed.

**b. Carbohydrates**

- **Anthrone test:** Take 2 ml of Anthrone test solution, adding 0.5 ml of hydro-alcoholic extract of the drug. A green or blue colour indicates the presence of carbohydrates.
- **Benedict's test:** Take 0.5 ml of hydro-alcoholic extract of the drug adding 5 ml of Benedict's solution and boiling for 5 minutes. Formation of a brick red colour precipitate is due to the presence of carbohydrates.
- **Fehling's test:** Take 2 ml of hydro-alcoholic extract of the drug adding 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boiling the contents of the test tube for few minutes. A red or brick red precipitate is formed.
- **Molisch's test:** In a test tube containing 2 ml of hydro-alcoholic extract of the drug adding 2 drops of a freshly prepared 20% alcoholic solution of  $\beta$ - naphthol and mix, pouring 2 ml conc. sulphuric acid so as to form a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

**c. Flavonoids**

- **Shinoda's test:** In a test tube containing 0.5 ml of hydro-alcoholic extract of the drug, adding 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

**d. Proteins**

- **Biuret's test:** To 1 ml of hot hydro-alcoholic of the drug adding 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.
- **Millon's test:** Dissolving a small quantity of hydro-alcoholic of the drug in 1 ml of distilled water and adding 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

**e. Saponins**

In a test tube containing about 5 ml of hydro-alcoholic extract of the drug adding a drop of sodium bicarbonate solution, shaking the mixture vigorously and leave for 3 minutes. Honeycomb like froth is formed.

**f. Steroids**

- **Liebermann-Burchard's test:** Adding 2 ml of acetic anhydride solution to 1 ml of hydro-alcoholic extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.
- **Salkowski Reaction:** Adding 1 ml of conc. sulphuric acid to 2 ml of hydro-alcoholic extract of the drug carefully from the side of the test tube. A red colour is produced in the chloroform layer.

**g. Tannins**

- To 1-2 ml of plant hydro-alcoholic extract, adding a few drops of 5% FeCl<sub>3</sub> solution was added. A green colour indicates the presence of gallo-tannins while brown colour tannins.

**h. Glycosides**

Detection of glycoside on paper spray solution No. 1 (0.5% aqueous sol. of Sodium metaperiodate) & waiting for 10 minutes after then spraying solution No. 2 [0.5% Benzidine (w/v) in solution of Ethanol-acetic Acid (4:1)], white spot with blue background shows presence of glycoside.

**6. TLC [2]**

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R<sub>f</sub> value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

**Preparation of plates** - Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100° to 105°

for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

**Method**

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualize as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

**R<sub>f</sub> Value**

Measure and record the distance of each spot from the point of its application and calculate the R<sub>f</sub> value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

1. 4 gm of sample was taken in 40 ml rectified spirit (90%)
2. Shaking it for 18 hrs & boiled for 10 mins & filtered.
3. The filtrate was then evaporated & extracted chloroform.
4. The soluble portion was filtered, concentrated & made upto 10 ml in flask.
5. The solution was applied on aluminium plate precoated with silica gel 60F254 of 0.2 mm thickness using linomat IV applicator. The plate was developed in toluene: Ethyl acetate (9.1), (3.1). After air drying the plate was visualized in UV-254 & 366nm. The plate was then dipped in Bromine fume & heated in hot air oven at 105°C till the spots appeared.

**7. UV-Vis spectroscopy [4]**

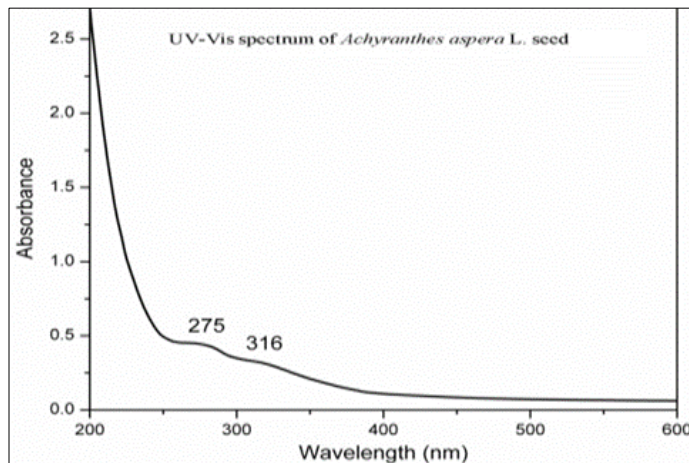
A variety of techniques can be used to determine and estimate the presences of phytoconstituents in medicinal plants. Spectroscopic methods have been firmly established as a key technological platform to identify and to characterize the biomolecules presents in medicinal plants. The phytoconstituents always resemble their fingerprints (unique signature) under spectroscopic characterizations viz. UV-Vis, Photoluminescence, FT-IR and Raman studies. Thus, the present study is focused to understand the UV-Vis characteristics of seeds of *Achyranthes aspera* L. as well as to identify the contained phytoconstituents. Among the spectroscopic techniques, ultraviolet-visible spectroscopy (UV-Vis) has become the most important analytical instrument in the modern day laboratory. It refers to

absorption or reflectance spectroscopy. UV-Vis spectrophotometry is related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses electromagnetic radiation in the visible ranges or its adjacent ranges. The color of the chemical(s) directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum. UV-Vis spectroscopic studies help in identification and authentication of the phytoconstituents of medicinal plants. This information may be act as reference for correct knowledge and also help as a tool to detect adulteration of that particular medicinal plant. In the present investigations,

the extract of *Achyranthus aspera* L. was subjected to phytochemical screening by using UV-Vis spectroscopic technique to identify phytoconstituents.

### Sample characterization

To record the UV-Vis spectra of extract of seeds of *Achyranthes aspera* L. the sample were scanned in the wavelength range 200–600 nm by using UV-Vis spectrometer (Perkin Elmer Lambda 25). UV-Vis spectrometer used for characterization is double beam spectrometer in which deuterium lamp is used for UV radiation and tungsten-halogen lamp is employed for visible (Vis.) radiation.



**Fig 5:** UV-Vis spectra of Seeds of *Achyranthes aspera* L.

Fig. 5 exhibit the UV-Vis spectra of samples *Achyranthes aspera* L. The UV-Vis profile sample showed the peaks at 275 nm with absorbance 0.49 and another peaks at 316 nm with absorbance 0.36. The results obtained in UV-Vis spectra

revealed the existence of several medicinally important phytoconstituents.

### Result and Discussion

**Table 1:** Certificate of analysis of seeds of *Achyranthes aspera* L.

Parameters	Observation
Nature	Coarse powder
Colour	Brown
Taste	Astringent
Foreign matter	2%
LOD	5.3%
Total ash value	8.25 (% w/w)
Acid insoluble ash	3.0 (% w/w)
pH	7.5

**Table 2:** Percentage yield of Extracts of seeds of *Achyranthes aspera* L.

Extracts	Nature of Extract	Weight (g)/100g of drug	% Yield w/v
Hydro-alcohol	Viscous	10.6	10.01

**Table 3:** Phytochemical screening of hydro-alcoholic extract of seeds of *Achyranthes aspera* L.

S No.	Chemical Tests	Hydroalcoholic Extract
	Carbohydrates	-
	Proteins	-
	Amino acids	-
	Glycosides	-
	Flavanoids	+
	Alkaloids	+
	Tannins	+
	Steroids	-
	Phenolic Compounds	+
	Coumarin	+
	Saponin	+
	Resin	+
	Starch	-

The hydro-alcoholic extract of seeds of *Achyranthes aspera* L. was prepared. A large number of solvent systems were tried to achieve a good resolution.

Rf Value = 0.13, 0.46, 0.58, 0.65, 0.81

### Conclusion

On the basis of the result of seeds *Achyranthes aspera* L. is a Ayurvedic medicinal plant. It had the two peaks in UV-Vis spectra sample showed the peaks at 275 nm with absorbance 0.49 and another peaks at 316 nm with absorbance.0.36 in fig.5. It was confirms the presence of tannins and flavonoids. By the Qulitative Phytochemical Screening Resin, Saponin, Coumarin, Phenolic Compounds, Flavanoids, Alkaloids, tannins are present. We found The Extracts of hydroalcoholic Percentage yield is 10.01%, four Rf value is found 0.13, 0.46, 0.58, 0.65, 0.81 and all parameter are within limit. The quality of drug is good.

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